PROCESS OF EXTRACTING SMALL MOLECULAR INGREDIENTS FROM BIOLOGICAL MATERIALS UNDER SUPER HIGH PRESSURE

Field of the invention

The present invention relates to a process of extracting from biological materials under super high pressure, especially to a process of extracting small molecular active ingredients from biological materials under super high pressure.

The biological materials include the total, partial, tissue, partial tissue or other parts of plants, animals and microorganisms.

The small molecules include the molecules that comprise fewer atoms with lower molecular weight. Generally, the molecular weight is lower than 10,000.

The active ingredients include the components that can be used for treatment of diseases, physiological accommodation, improvement of health, promotion of growth, as well as improvement of color, flavour and taste of food, etc.. Natural produces comprise many kinds of active ingredients, such as alkaloids, glucosides, organic acids, volatile oils, terpenoids, flavonoids, steroids, oligosaccharides, polysaccharides, coumarins, lignins, saponin, amino acids, peptides and proteins, enzymes, tannins, resin, pigments of plant, oils, wax, inorganic salts, etc.. These active ingredients are generally divided into hydrophilic and lipophilic substance according to their solubility. Many ingredients will be inactive or active lowered after heating.

The super high pressure, said in the invention, is hydrostatic pressure of 100MPa ~ 1000MPa.

Background of the invention

There are many methods of extracting active ingredients from biomaterials. The methods for extracting active ingredients from natural products are solvent exaction (including immersion, leaching, refluxing, boiling), vapour distillation and sublimation, Gao Yuanjun, The development and processing of the wild plants in China, Chinese light industry press, Beijing, 1997.02. The methods to extract

natural components are the solvent technology, vapour distillation, and sublimation. The latter two are limitedly used, the solvent technology is used in most conditions, Yao xinsheng, Chemistry of natural drugs, People's Medical Publishing House, Beijing, 1998.06. These methods have many advantages, for example, many kinds of solvent can be used, many active ingredients can be obtained and the extraction yield is higher. However, there are also many shortages, for example, difficulty of separating and purifying, largely denaturing of the active ingredients, much loss of the ingredient by volatilization, and long extraction time, etc. Although several technologies have been developed recently, such as enzyme technology, ultra-micro-pulverizing technology etc., the above shortages are not be eradicated.

The newest technology supercritical CO_2 extraction has solved some of the above problems, particularly has a notable advantage of separation and purification, and accordingly has become a very important extraction technology. However, Zhu Zhiqiang described in Supercritical fluid technology, principle and application, Chemical industry press, Beijing, 2000.03: CO_2 has lipophilic property. The fragrant oils, fat, alcohol, aldehyde, ketone, wax, the light fraction of resin can be selectively extracted by supercritical CO_2 , ……the saccharides and salts are insoluble in supercritical CO_2. The solubility of alkaloids, polysaccharides, steroids, terpenoids, glycosides, saponin or flavonoids, etc. in supercritical CO_2 is very poor, which makes it difficult for these substances to be extracted by supercritical CO_2 exaction. Additionally, alkaloids, nicotine and caffeine may react with other components, such as citric acid and caffetannic acid, to form salts, which causes it difficult to extract these ingredients by supercritical CO_2. In addition, there are other disadvantages of supercritical CO_2 extraction, for example, expensive equipment and high cost of operation.

Description of the invention

This invention provides a new process of extracting small molecular ingredients from biomaterials under super high pressure with high extraction yield and short extraction time to overcome the disadvantages of present extraction

methods.

The process of extracting small molecular ingredients from biomaterials according to the invention is a process of extracting small molecular ingredients form the mixture of the solid raw biomaterial and solvent under super high pressure. The procedures are as follows:

The step of pretreatment, crashing and formulation: the solid raw biomaterial is pretreated first, including roguing, cleaning, or macerating, and then crashed. Finally mix the crashed biomaterials with proper solvent by the proper ratio homogeneously.

The step of closure: put the above mixture into the high pressure container firstly and then close the container. Alternatively, close the container firstly and then charge the mixture into the container.

The step of increasing pressure: The pressure of the pressure container is increased from normal pressure to the predefined pressure of 100MPa ~ 1000MPa.

The step of holding pressure: The predefined pressure is held for 3-30 minutes.

The step of releasing pressure: The pressure of the pressure container is released to the normal pressure, and the mixture is removed from the container.

Alternatively, in the step of closure, the mixture is first poured into a packing container which is then airproofed, after that, put the packing container into the pressure container and close it. Charge the medium for transferring pressure into the pressure container. The pressure of the pressure container is increased via the medium by a pump. After holding for a period of time, release the pressure of container, and remove the packing container and the mixture therein. The packing container may be made of flexible material, such as plastic film or paper. It also may be made of hard materials, such as metal or glass. The packing container must be pressure transferrable without breakage, osmosis and leakage under super high pressure. Additionally, it does not react with solvent, raw biomaterials and medium for transferring pressure.

The steps of increasing pressure, holding pressure and releasing pressure can be finished by one step or several steps. If finished by several steps, it can be done as

the following:

① To increase the pressure in a ladder-type: increasing pressure in a ladder-type means that pressure is increased to the first predefined pressure, and hold for certain period time, then the pressure is increased to the second predefined higher pressure, and hold certain period time again, after that the pressure is increased again, thus repeat until reach the highest predefined pressure, and hold for certain time, then release the pressure.

② To increase pressure in a pulse-type: increasing pressure in a pulse-type means that the pressure is increased to the first predefined pressure, and hold for certain period time, release the pressure. The pressure is increased again to the second predefined pressure, hold for certain period time again and release the pressure. Thus repeat several times. The pressure reached each time may be same or not. The mixture is removed from the container after releasing the pressure at last time.

③ The raw biomaterial may be extracted one time or several times, i.e., the raw material which has been extracted under super high pressure is mixed with solvent and extracted once again under super high pressure. Such extraction can be repeated several times and the solvent can be the same or not each time.

One or more devices may be placed in the pressure container so that the super high pressure can work combined with other processing technologies. The devices may be ultrasonator, electric pulser, machine stirrer, heater, or cooler, etc. Such devices can work at any step, several steps or total steps of before increasing pressure, increasing pressure, holding pressure, releasing pressure or after releasing pressure.

The above devices may also be used outside the pressure container before extraction. At such circumstance, the processing technologies by use of the above devices are called prior treatment.

The extraction solvent of the invention includes water and/or organic solvent, or the mixture thereof. The organic solvent may include alcohols (for example, methanol, ethanol, isopropyl alcohol, butanol, etc.), ether (for example, dioxane,

diethyl ether, petroleum ether, etc.), alkane halide (for example, chloroform, dichloromethane, etc.), ketone (for example, acetone, etc.), hydrocarbon (for example, hexane, industrial solvent oil, etc.), acids (for example, acetic acid, fatty acid etc.), amine (for example, ethanol amine, etc.), and the mixture thereof.

The medium for transferring pressure of the invention is liquid, which is the same or not with extraction solvent.

At the step of pretreatment, crashing and formulation, some chemicals and/or bio-products as auxiliary additives may be added into the mixture of raw material and solvent. The auxiliary additive added may be one or more kinds. The auxiliary additives may be act as cosolvent, restrainer, precipitating agent or reactant to improve the performance of extraction under super high pressure, or to change structures of the extract.

During extraction under super high pressure, the temperature may be increased or reduced. The medium for transferring pressure and/or the mixture of raw material and solvent are heated with heater and cooled with cooler. The heater and/or cooler are sometimes placed or assembled in the pressure container. Sometimes, the pressure container is put into cooler to cool the mixture and/or medium.

The advantages of the invention are as follows:

A. The extraction solvent has broad scope, including water and /or organic solvent and the mixture thereof. Therefore, the most proper solvent can be selected as extraction solvent. The present invention has the advantage of using much more kinds of solvent over supercritical CO_2 extraction.

B. The extraction under high pressure is operated at room temperature during which the change of temperature is within 5 ℃ except of being heated or cooled. So, the extraction process according to the present invention has not the shortages of reaction, denaturation, or loss of the active ingredients which appeared in the common heat extraction such as refluxing, immersion, leaching, boiling and distilling. Thus, it is very useful for extracting heat sensitive ingredients. Sometimes, the extraction under high pressure is combined with heating or cooling to improve the performances of extraction, such as increasing the extract yield, changing the

ingredients of extract, etc..

C. Theoretically, the solubility of most compounds increases with the increase of pressure. The pressure of the extraction of the present invention is above 100MPa which is far higher than that of the common extraction and supercritical CO_2 extraction (usually about 10 MPa). Thus, the solubility of the bioactive ingredients is much better and the extract yield is much higher accordingly.

D. Under super high pressure, the cell membranes are ruptured, and the active ingredients in the cell can easily enter into the solvent, and the solvent also can easily enter into the cell. Thus, the extraction time is greatly shortened. Using the routine methods of extraction, the time of extraction is much longer. In general, the time of cool immersion and enzymolysis is from one day to several days; the extraction time of refluxing, boiling or distilling is 6~8 hour; and the extraction time of supercritical CO_2 is about 2 hour or more. However, the extraction time under super high pressure is generally 2~15 minutes, and the maximum is no more than 30 minutes.

E. Under super high pressure, the protein and starch are denatured, but not cleaved, which makes separation and purification more simple and convenient.

F. The process according to the invention can be used to extract many kinds of active ingredients. The medium for transferring pressure can be the same or not with the solvent. The pressure equipment is easy to be operated and controlled. Furthermore, the pressures everywhere of the pressure container are equivalent, which makes the extraction conditions constant. The pressure equipment can be used for extracting various kinds of active ingredients with various kinds of solvents.

G. Because the solubility of ingredients increases under super high pressure, less solvent is needed. The pollutant released is greatly decreased than other extraction methods.

H. Energy is saved in the process of the invention. One reason is that there is no energy consumption at the step of holding pressure. Additionally the compressibility of liquid is less, and the energy to compress liquid is far less than that to raise

temperature and to compress CO_2 into supercritical state.

Brief description of the drawings

Fig. 1 is the sketch of extracting from the mixture of raw material and solvent in a packing container under high pressure via medium for transferring pressure.

Fig. 2 is the sketch of extracting directly from the mixture of raw material and solvent under high pressure.

Fig. 3 is the sketch of extracting from the mixture of raw material and solvent under high pressure by the movement of piston.

Fig. 4 is the sketch of a pressure container of Fig 1 containing other devices.

Fig. 5 is the sketch of a pressure container of Fig 2 containing other devices.

Fig. 6 is the flow chart of extracting under super high pressure.

Detailed description of the embodiments

The details of the process of the invention are further explained with reference to the drawing as following:

Fig. 6, the flow chart of extracting under super high pressure shows that the procedures of the process of extracting activity ingredient are as the following: The step of pretreatment, crashing and formulation (S1) is that the solid raw biomaterial is pretreated first, including roguing, cleaning, or macerating, and then is crashed, inflated, or homogenized, and mixed with solvent by the proper ratio. If the auxiliary additive is required, it can be added during mixing the raw material and solvent. If the prior treatment is required, it should works at this step. Finally, charge the mixture into the packing container and make it airtight. The step of closure (S2) is: put the formulated mixture of step (S1) into the pressure container firstly, and then close the container. Either way, the mixture is poured into a packing container, which is then airproofed, and finally put the packing container into the pressure container and close it, and after that, the medium for transferring pressure is charged into. The step of increasing pressure (S3) is that the pressure of the pressure container is increased from normal pressure to the predefined pressure of 100MPa ~ 1000MPa at room temperature. The step of holding pressure (S4) is that

the predefined pressure is held for 3-30 minutes. The step of releasing pressure (S5) is that the above predefined pressure is released to the normal pressure, the container is opened and the mixture is removed therefrom.

The steps drawn by dotted line in Fig. 6 can be used totally, or partially, or not used at all according to the raw materials or the active ingredients.

As shown in Fig. 1, the mixture of raw material and solvent (5) is put into a packing container (4) which is then airproofed, and then the container (4) is put into the high pressure container (1) which is then closed with the end-lid (2). The medium for transferring pressure (6) is introduced into the container (1) through the connecting tube line (3). The medium (6) is pressed by pump or supercharger to increase the pressure of container (1) to the predefined pressure of 100MPa~1000MPa. The predefined pressure is held for 3-30 minutes, and then released to normal pressure. The end-lid (2) is removed, and the packing container (4) is taken from the container (1), and then the mixture of raw material and solvent (5) is removed from the container (4).

As shown in Fig. 2, the mixture of raw material and solvent (5) is charged into the high pressure container (1) and then the container (1) is closed with the end-lid (2). The pressure of container (1) is increased to the predefined pressure of 100MPa~1000MPa by pump or supercharger. After that, the steps of holding pressure and releasing pressure proceed. Alternatively, the pressure container (1) is closed with lid (2) firstly, and then the mixture (5) is introduced into the container (5) through connecting tube (3) by pump or supercharger. After that, steps of increasing pressure, holding pressure and releasing pressure proceed.

As shown in Fig. 3, the mixture of raw material and solvent (5) is charged into the pressure container (1), but the end-lid (2) of Fig. 2 is replaced by the piston (7). The steps of increasing pressure, holding pressure and releasing pressure are accomplished by the movement of piston (7).

As shown in Fig. 4, the device (8) is placed or assembled in the high pressure container (1) of Fig 1, wherein the mechanical stirrer is not included in the device (8).

As shown in Fig. 5, the device (8) is placed or assembled in the high pressure container (1) of Fig 2, wherein the mechanical stirrer is included.

EXAMPLES

Example 1

To extract *flavonoids* from the gingko leaves:

Take off the impurities in gingkgo leaves, and crash the gingko leaves into powder with a pulverizer, and then mix 1g powder with 100ml water. The mixture is sealed into a plastic film bag which is then put into the high pressure container. Close the container, and then introduce the mixture of kerosene and transformer oil as medium for transferring pressure. Increase the pressure of the pressure container to 500MPa via the medium by a supercharger. Hold this pressure for 10 minutes, and then release it to normal pressure. The container is opened and the mixture is removed from the bag.

The comparing results of extracting under super high pressure and the routine extraction technologies are shown as following:

No.	Formulated mixture	Extraction technology	The content of *flavonoids* in the fluid extract
1	1g gingko leaves + 100ml water	Super high pressure 500MPa, 10 minutes	3.10 mg/ml
2	1g gingko leaves + 100ml water	Boiling (1 hour)	2.43 mg/ml

Example 2

To extract the *tea polyphenols* from the tea leaves

Style 2: Take off the impurities in tea leaves and crash tea leaves into powder. Mix 3g powder of tea leaves with 540ml water and 0.5ml of 75% ethanol as the auxiliary additive. The above mixture is sealed into a plastic film bag which is then put into the pressure container. Close the container, and then introduce the mixture of kerosene and transformer oil as medium for transferring pressure. Increase the pressure of the pressure container to 460MPa via the medium by a supercharger.

Hold this pressure for 10 minutes, and then release it to normal pressure. The container is opened and the mixture is removed from the bag.

Style 3: Take off the impurities in tea leaves and crash the tea leaves into powder. Mix 3g powder of tea leaves with 360ml of 80% ethanol. The above mixture is sealed into a plastic film bag which is then put into the pressure container. Close the container, and then introduce water as the medium for transferring pressure. Increase the pressure of the pressure container to 380MPa via the medium by a supercharger. Hold this pressure for 10 minutes, and then release it to normal pressure. The container is opened and the mixture is removed from the bag.

The results, obtained by the extraction technology under super high pressure and the routine extraction technologies are shown respectively as following:

No.	Formulated mixture and Extraction technology	The content of tea *polyphenols* in the fluid extract
1	3g tea leaves + 500ml water Boiling, 1 hour	24.0 mg/ml
2	3g tea leaves + 540ml water Super high pressure of 460 MPa, 10 minutes	18.68 mg/ml
3	3g tea leaves + 360ml 80% ethanol Super high pressure 380 MPa, 10 minutes	28.0 mg/ml

Example 3

To extract *Baicalein* from *Radix Scutellariae*

Take off the impurities in *Radix Scutellariae*; and crash the *Radix Scutellariae* with pulverizer. Mix 5g powder of *Radix Scutellariae* with 100ml of 60% methanol. Treat the mixture for 10 minutes with a ultrasonator. The above mixture is sealed into a plastic film bag which is immersed for 4 hours at room temperature. Put the bag into the pressure container, and close the container. Then introduce the mixture of kerosene and transformer oil as medium for transferring pressure. Increase the

pressure of the pressure container to 200MPa via the medium by a supercharger. Hold this pressure for 5 minutes, and then release it to normal pressure. Increase the pressure of the pressure container to 600MPa. Hold this pressure for 5 minutes, and then release it to normal pressure. The container is opened and the mixture is removed from the bag.

The results, obtained by the extraction technology under super high pressure and the routine extraction technologies are shown respectively as following:

No.	Formulated mixture	Extraction technology	The content of Baicalein in the fluid extract
1	5g *Radix Scutellariae* + 100ml methanol	Under super high pressure	3.10 mg/ml
2	5g *Radix Scutellariae* + 100ml methanol	Heat till reflux at 100℃, 2.5 h.	1.23 mg/ml

INDUSTRIAL APPLICABILITY

The process for extracting small molecular ingredients from biological materials under super high pressure according to the invention is usually operated at room temperature. Thus, the denatured and loss of the active ingredients caused by heating are avoided. A large range of solvents can be used in this technology to extract the hydrophilic and lipophilic active ingredients. Additionally, it has advantages such as shorter extraction time, higher extract yield, easier to be separated and purified. It is a fast extraction technology with high-yield for small molecular active ingredients.